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BAT CORONAVIRUSES

Xing-Yi Ge, Ben Hu, and Zheng-Li Shi

Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Hubei, China

5.1 INTRODUCTION

Bats have been documented as natural hosts of human diseases since the beginning of the 20th century (Courter, 1954). In the last 40 years, several emerging human viral infections have been linked to bats, including lyssavirus (Samaratunga *et al.*, 1998; Stantic-Pavlinic, 2005), Ebola virus (Leroy *et al.*, 2005), Hendra virus (Halpin *et al.*, 1999), and Nipah virus (Chua *et al.*, 2002). Coronaviruses were not detected in bats until 2005, just after the outbreak of severe acute respiratory syndrome (SARS) (Lau *et al.*, 2005; Li F *et al.*, 2005b; Poon *et al.*, 2005). Uncovering the origin of the SARS coronavirus led to the discovery of coronaviruses in bats. Since then a variety of coronaviruses have been identified in more than 100 bat species distributed throughout Asia, Australia, Europe, Africa, and America (Shi, 2013; Chen *et al.*, 2014) (Table 5.1). Fifty-nine full-length genomes of bat coronaviruses have been sequenced to date, and partial coronavirus genomic sequences deposited in GenBank have exceeded 600 (Chen *et al.*, 2014).

Coronaviruses belong to the subfamily *Coronavirinae*, family *Coronaviridae*, a monophyletic cluster in the order *Nidovirales* (de Groot *et al.*, 2012). The coronavirus genome has a positive-sense single-stranded RNA of 26–32 kb in size, the largest

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TABLE 5.1 Coronaviruses detected in bats

Bat family and species	Bat CoV species	Country	Genomic sequence	Reference
Vespertilionidae				
Eptesicus fuscus	Unclassified alphacoronavirus	USA	Partial	Donaldson *et al.*, 2010; Huynh *et al.*, 2012; Osborne *et al.*, 2011
Eptesicus isabellinus	Unclassified betacoronavirus	Spain	Partial	Falcon *et al.*, 2011
Eptesicus serotinus	Unclassified betacoronavirus	Italy	Partial	De Benedictis *et al.*, 2014
Eptesicus spp.	Unclassified alphacoronavirus	Mexico	Partial	Anthony *et al.*, 2013b
Hypsugo savii	Unclassified alphacoronavirus	Spain	Partial	Falcon *et al.*, 2011
	Unclassified betacoronavirus	Italy	Partial	Lelli *et al.*, 2013
Miniopterus africanus	Unclassified coronavirus	Kenya	Partial	Tong *et al.*, 2009
Miniopterus australis	Unclassified alphacoronavirus	Australia	Partial	unpublished
Miniopterus fuliginosus	Unclassified alphacoronavirus	Japan	Partial	Shirato *et al.*, 2012
Miniopterus inflatus	Unclassified alphacoronavirus	Kenya	Partial	Tao *et al.*, 2012; Tong *et al.*, 2009
Miniopterus magnater	Alphacoronavirus 1A	China	Full length	Chu *et al.*, 2008
	Alphacoronavirus HKU7	China	Partial	Chu *et al.*, 2006; Woo *et al.*, 2006
	Alphacoronavirus HKU8	China	Full length	Chu *et al.*, 2008; Chu *et al.*, 2006
Miniopterus natalensis	Unclassified alphacoronavirus	Kenya	Partial	Tong *et al.*, 2009
Miniopterus pusillus	Alphacoronavirus 1B	China	Full length	Chu *et al.*, 2008; Chu *et al.*, 2006; Poon *et al.*, 2005
	Alphacoronavirus HKU8	China	Partial	Woo *et al.*, 2006
Miniopterus schreibersii	Unclassified alphacoronavirus	Spain,	Partial	Falcon *et al.*, 2011
	Alpacoronavirus 1B	China	Partial	Tang *et al.*, 2006; Vijaykrishna *et al.*, 2007
	Unclassified alphacoronavirus	Bulgaria	Partial	Drexler *et al.*, 2010
	Unclassified alphacoronavirus	Australia	Partial	unpublished
Miniopterus spp.	Unclassified coronavirus	Kenya	Partial	unpublished
	Unclassified coronavirus	South Africa	Partial	Geldenhuys *et al.*, 2013
Myotis bechsteini	Unclassified alphacoronavirus	Germany	Partial	Gloza-Rausch *et al.*, 2008
Myotis blythii	Unclassified alphacoronavirus	Spain	Partial	Falcon *et al.*, 2011

Myotis dasycneme	Unclassified alphacoronavirus	Germany	Partial	Gloza-Rausch *et al.*, 2008
	Unclassified betacoronavirus	Netherlands	Partial	Reusken *et al.*, 2010
Myotis daubentonii	Unclassified alphacoronavirus	Germany	Partial	Gloza-Rausch *et al.*, 2008
	Unclassified alphacoronavirus	United Kingdom	Partial	August *et al.*, 2012
	Unclassified betacoronavirus	The Netherlands	Partial	Reusken *et al.*, 2010
	Unclassified coronavirus	China	Partial	He *et al.*, 2014
	Unclassified alphacoronavirus	Spain	Partial	Falcon *et al.*, 2011
	Unclassified coronavirus	Germany	Partial	Drexler *et al.*, 2010
Myotis davidii	Unclassified coronavirus	China	Partial	He *et al.*, 2014
Myotis lucifugus	Unclassified alphacoronavirus	USA	Full length	unpublished
	Unclassified alphacoronavirus	USA	Partial	Dominguez *et al.*, 2007
Myotis macropus	Unclassified alphacoronavirus	Australia	Partial	unpublished
Myotis myotis	Unclassified alphacoronavirus	Spain	Partial	Falcon *et al.*, 2011
	Unclassified coronavirus	Germany	Partial	Drexler *et al.*, 2011
Myotis nattereri	Unclassified alphacoronavirus	United Kingdom	Partial	August *et al.*, 2012
Myotis ricketti	Alphacoronavirus HKU6	China	Partial	Woo *et al.*, 2006
Myotis rufus	Unclassified alphacoronavirus	Brazil	Partial	Goes *et al.*, 2013
Myotis velifer	Unclassified alphacoronavirus	Mexico	Partial	Anthony *et al.*, 2013b
Myotis volans	Unclassified alphacoronavirus	USA	Partial	Osborne *et al.*, 2011
Neoromicia cf. zuluensi	Unclassified betacoronavirus	South Africa	Partial	Ithete *et al.*, 2013
Neoromicia spp.	Unclassified coronavirus	South Africa	Partial	Geldenhuys *et al.*, 2013
Nyctalus lasiopterus	Unclassified alphacoronavirus	Spain	Partial	Falcon *et al.*, 2011
Nyctalus leisleri	Unclassified alphacoronavirus	Bulgaria	Partial	Drexler *et al.*, 2010
Nyctalus noctula	Unclassified betacoronavirus	Italy	Partial	Lelli *et al.*, 2013
	Unclassified betacoronavirus	Netherlands	Partial	Reusken *et al.*, 2010
Pipistrellus abramus	Betacoronavirus HKU5	China	Full length	Woo *et al.*, 2007
	Betacoronavirus HKU5	China	Partial	Lau *et al.*, 2013; Woo *et al.*, 2006
Pipistrellus kuhlii	Unclassified alphacoronavirus	Spain	Partial	Falcon *et al.*, 2011; Lelli *et al.*, 2013
	Unclassified alphacoronavirus	Saudi Arabia	Partial	Memish *et al.*, 2013
	Unclassified betacoronavirus	Italy	Partial	Lelli *et al.*, 2013

(*Continued*)

TABLE 5.1. (*Continued*)

Bat family and species	Bat CoV species	Country	Genomic sequence	Reference
Pipistrellus nathusii	Unclassified betacoronavirus	Romania	Partial	Annan *et al.*, 2013
	Unclassified betacoronavirus	Ukraine	Partial	Annan *et al.*, 2013
	Unclassified alphacoronavirus	Germany	Partial	Gloza-Rausch *et al.*, 2008
Pipistrellus pipistrellus	Unclassified betacoronavirus	Netherlands	Partial	Reusken *et al.*, 2010
Pipistrellus pygmaeus	Unclassified betacoronavirus	Romania	Partial	Annan *et al.*, 2013
	Unclassified alphacoronavirus	Germany	Partial	Gloza-Rausch *et al.*, 2008
Pipistrellus spp.	Unclassified alphacoronavirus	Spain	Partial	Falcon *et al.*, 2011
Pipistrellus subflavus	Unclassified alphacoronavirus	USA	Partial	Huynh *et al.*, 2012
Scotoecus spp.	Unclassified coronavirus	Kenya	Partial	Tong *et al.*, 2009
Scotophilus kuhlii	Unclassified alphacoronavirus	Philippines	Partial	Watanabe *et al.*, 2010
	Bat alphacoronavirus 512	China	Full length	Tang *et al.*, 2006
Tylonycteris pachypus	Betacoronavirus HKU4	China	Full length genome	Lau *et al.*, 2013; Tang *et al.*, 2006; Woo *et al.*, 2007
Vespertilio superans	Unclassified bat betacov/SC2013	China	Full length	Yang *et al.*, 2014
Rhinolophidae				
Rhinolophus affinis	Bat SARS-related coronavirus	China	Full length	He *et al.*, 2014
Rhinolophus blasii	Bat SARS-related coronavirus	Bulgaria	Full length	Drexler *et al.*, 2010
	Unclassified alphacoronavirus	Bulgaria	Partial	Drexler *et al.*, 2010
Rhinolophus cornutus	Unclassified betacoronavirus	Japan	Partial	unpublished
Rhinolophus euryale	Alphacoronavirus 1B	Bulgaria	Partial	Drexler *et al.*, 2010
	Bat SARS-related coronavirus		Partial	Drexler *et al.*, 2010
Rhinolophus ferrumequinum	Unclassified alphacoronavirus	Bulgaria	Partial	Drexler *et al.*, 2010
	Bat SARS-related coronavirus	Bulgaria	Partial	Drexler *et al.*, 2010
	Bat SARS-related coronavirus	China	Full length	Li W *et al.*, 2005a; Tang *et al.*, 2006
	Unclassified alphacoronavirus	China	Partial	Tang *et al.*, 2006; Vijaykrishna *et al.*, 2007
	Unclassified alphacoronavirus	China	Partial	He *et al.*, 2014
Rhinolophus hipposideros	Unclassified coronavirus	Slovenia	Partial	Rihtaric *et al.*, 2010
	Unclassified betacoronavirus	Italy	Partial	Lelli *et al.*, 2013
	Unclassified alphacoronavirus	China	Partial	He *et al.*, 2014

Rhinolophus landeri	Unclassified coronavirus	Kenya	Partial	unpublished
Rhinolophus macrotis	Bat SARS-related coronavirus	China	Full length	Li *et al.*, 2005; Tang *et al.*, 2006
Rhinolophus megaphyllus	Unclassified alphacoronavirus	Australia	Partial	unpublished
Rhinolophus mehelyi	Bat SARS-related coronavirus	Bulgaria	Partial	Drexler *et al.*, 2010
Rhinolophus pearsonii	Bat SARS-related coronavirus	China	Full length	Li *et al.*, 2005
Rhinolophus pusillus	Bat SARS-related coronavirus	China	Full length genome	Yang *et al.*, 2013
Rhinolophus sinicus	Bat SARS-related coronavirus	China	Full length	Ge *et al.*, 2013; Lau *et al.*, 2005; Li *et al.*, 2005; Ren *et al.*, 2006; Yuan *et al.*, 2010
	Alphacoronavirus HKU2	China	Full length	Lau *et al.*, 2007; Woo *et al.*, 2006
Rhinolophus spp.	Unclassified coronavirus	Kenya	Partial	unpublished
	Unclassified coronavirus	Rwanda	Partial	unpublished
Rhinonicteris aurantia	Unclassified betacoronavirus	Australia	Partial	unpublished
Rhinopomatidae				
Rhinopoma hardwickei	Unclassified alphacoronavirus	Saudi Arabia	Partial	Memish *et al.*, 2013
Hipposideridae				
Hipposideros armiger	Unclassified alphacoronavirus	Thailand	Partial	unpublished
	Betacoronavirus HKU10	China	Partial	Ge *et al.*, 2012
Hipposideros caffer	Unclassified betacoronavirus	Gabon	Partial	unpublished
Hipposideros commersoni	Unclassified coronavirus	Kenya	Partial	Tong *et al.*, 2009
	Unclassified betacoronavirus	Nigeria	Partial	Quan *et al.*, 2010
Hipposideros diadema	Unclassified alphacoronavirus	Philippines	Partial	Tsuda *et al.*, 2012
Hipposideros larvatus	Unclassified coronavirus	Thailand	Partial	unpublished
Hipposideros pomona	Alphacoronavirus HKU10	China	Full length	Lau *et al.*, 2012
Hipposideros spp.	Unclassified alphacoronavirus	Ghana	Partial	Pfefferle *et al.*, 2009
Pteropodidae				
Cynopterus brachyotis	Unclassified betacoronavirus	Philippines	Partial	Watanabe *et al.*, 2010
Epomophorus labiatus	Unclassified coronavirus	Kenya	Partial	unpublished
Eidolon helvum	Unclassified betacoronavirus	Kenya	Partial	Tao *et al.*, 2012; Tong *et al.*, 2009
Micropteropus pusillus	Unclassified coronavirus	Central African Republic	Partial	unpublished

(*Continued*)

TABLE 5.1. (*Continued*)

Bat family and species	Bat CoV species	Country	Genomic sequence	Reference
Ptenochirus jagori	Unclassified betacoronavirus	Philippines	Partial	Tsuda *et al.*, 2012
Pteropus giganteus	Unclassified beta- and gammacoronavirus	Bangladesh	Partial	Anthony *et al.*, 2013a
Rousettus aegyptiacus	Unclassified coronavirus	Kenya	Partial	Tong *et al.*, 2009
Rousettus leschenaultii	Betacoronavirus HKU9	China	Full length	Lau *et al.*, 2010; Woo *et al.*, 2007
	Alphacoronavirus HKU10	China	Full length	Lau *et al.*, 2012
Phyllostomidae				
Anoura geoffroyi	Unclassified coronavirus	CostaRica	Partial	Corman *et al.*, 2013
Artibeus jamaicensis	Unclassified coronavirus	Panama	Partial	Corman *et al.*, 2013
	Unclassified alphacoronavirus	Mexico	Partial	Anthony *et al.*, 2013b
Artibeus lituratus	Unclassified coronavirus	Panama	Partial	Corman *et al.*, 2013
	Unclassified betacoronavirus	Mexico	Partial	Anthony *et al.*, 2013b
Artibeus phaeotis	Unclassified alphacoronavirus	Mexico	Partial	Anthony *et al.*, 2013b
Carollia brevicauda	Unclassified coronavirus	Brazil	Partial	Corman *et al.*, 2013
Carollia perspicillata	Unclassified alphacoronavirus	Trinidad	Partial	Carrington *et al.*, 2008
	Unclassified betacoronavirus	CostaRica	Partial	Corman *et al.*, 2013
	Unclassified coronavirus	Brazil	Partial	Corman *et al.*, 2013
	Unclassified alphacoronavirus	Mexico	Partial	Anthony *et al.*, 2013b
Carollia sowelli	Unclassified alphacoronavirus	Mexico	Partial	Anthony *et al.*, 2013b
Glossophaga soricina	Unclassified alphacoronavirus	Trinidad	Partial	Carrington *et al.*, 2008
Lonchorhina aurita	Unclassified alphacoronavirus	Mexico	Partial	Anthony *et al.*, 2013b
Phyllostomus discolor	Unclassified coronavirus	Panama	Partial	Corman *et al.*, 2013
Molossidae				
Chaerephon plicata	Bat SARS-related coronavirus	China	Full length	Yang *et al.*, 2013
Chaerephon pumilus	Unclassified alphacoronavirus	Kenya	Partial	Tong *et al.*, 2009
Chaerephon spp.	Unclassified alphacoronavirus	Kenya	Partial	Tong *et al.*, 2009
Molossus currentium	Unclassified coronavirus	Brazil	Partial	Corman *et al.*, 2013

Molossus rufus	Unclassified coronavirus	Brazil	Partial	Corman *et al.*, 2013
Mops midas	Unclassified coronavirus	South Africa	Partial	Geldenhuys *et al.*, 2013
Nyctinomops laticaudatus	Unclassified betacoronavirus	Mexico	Partial	Anthony *et al.*, 2013b
Otomops martiensseni	Unclassified coronavirus	Kenya	Partial	Tong *et al.*, 2009
Tadarida brasiliensis	Unclassified alphacoronavirus	Mexico	Partial	Anthony *et al.*, 2013b
Nycteridae				
Nycteris	Unclassified betacoronavirus	Ghana	Partial	Annan *et al.*, 2013
Emballonuridae				
Taphozous perforatus	Unclassified betacoronavirus	Saudi Arabia	Partial	Memish *et al.*, 2013
Mormoopidae				
Pteronotus davyi	Unclassified betacoronavirus	Mexico	Partial	Goes *et al.*, 2013
Pteronotus parnellii	Unclassified betacoronavirus	CostaRica	Partial	Corman *et al.*, 2013
	Unclassified betacoronavirus	Mexico	Partial	Anthony *et al.*, 2013b
Megadermatidae				
Cardioderma cor	Unclassified alphacoronavirus	Kenya	Partial	Tong *et al.*, 2009
Mystacinidae				
Mystacina tuberculata	Unclassified alphacoronavirus	New Zealand	Partial	Hall *et al.*, 2014

genome among the RNA viruses. The virions are 120–160 nm in diameter, spherical, and enveloped. The name of coronavirus was inspired by the equidistribution of the spike glycoproteins on the virion surface when viewed under the electron microscope giving the viral particle the appearance of the solar corona (Lai *et al.*, 2007). In the latest release of Virus Taxonomy by the International Committee on Virus Taxonomy (ICTV) in 2013, the *Coronavirinae* subfamily has four defined genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and the new genus *Deltacoronavirus* (de Groot *et al.*, 2012; ICTV, 2013). Among the classified coronavirus species, 15 belong to *Alphacoronavirus* and *Betacoronavirus* and mainly infect mammals, including humans, pigs, cats, and bats; two belong to *Gammacoronavirus* and only infect birds; while two belong to *Deltacoronavirus* and infect marine mammals. Remarkably, among the 15 classified *Alphacoronavirus* and *Betacoronavirus* species, 8 are from bats (Table 5.2). It has been suggested that bats are ideal hosts for both alphacoronaviruses and betacoronaviruses and may play an important role in the ecology and evolution of coronaviruses (Woo *et al.*, 2012).

In this chapter we provide an overview of the discovery and history of bat coronaviruses with a focus on SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV), both of which resulted in pandemics. We then turn to the genetic diversity of bat coronaviruses and discuss the taxonomic position and potential risk these coronaviruses pose to humans and other animals.

5.2 HUMAN DISEASES RELATED TO BAT CORONAVIRUSES

Coronaviruses usually cause mild respiratory symptoms in humans. Prior to the outbreak of SARS only two coronaviruses were known to infect humans: hCoV-229E and hCoV-OC43 (Vetterlein & Hesse, 1965; McIntosh *et al.*, 1967). Since then, four additional coronaviruses have been discovered in human patients: SARS-CoV (Falsey & Walsh, 2003), human coronavirus hCoV-NL63 (van der Hoek *et al.*, 2004), human coronavirus hCoV-HKU1 (Woo *et al.*, 2005), and MERS-CoV (Zaki *et al.*, 2012). SARS-CoV and MERS-CoV are highly pathogenic in humans and cause severe acute respiratory distress, with a high rate of mortality. Remarkably, both viruses are believed to have originated from bats.

5.2.1 SARS

5.2.1.1 SARS and SARS-CoV SARS, also known as infectious atypical pneumonia, is a novel emerging infectious disease that caused the first global pandemic of the 21st century (Zhong *et al.*, 2003). In November 2002, the first case of SARS was recorded in Foshan city, Guangdong Province, China (Chinese SARS Molecular Epidemiology Consortium, 2004; Song *et al.*, 2005). The disease spread rapidly to Beijing, Shanxi, Hong Kong, and other Provinces and regions across China. By July 2003, SARS had spread to 28 countries throughout the globe. According to the World Health Organization (WHO) there were 8096 reported cases and 774 deaths (WHO, 2004a). In that same year a novel coronavirus called SARS-CoV was isolated and identified to be responsible for the pandemic (Drosten *et al.*, 2003; Ksiazek *et al.*, 2003; Peiris *et al.*, 2003).

TABLE 5.2 Classified species and prototype strains in coronavirus genera

Species	GenBank accession No. of representative strains	Host	Reference
Genus *Alphacoronavirus*			
Alphacoronavirus 1			
Canine coronavirus	GQ477367	Dog	Herrewegh *et al.*, 1998
Feline coronavirus type I	EU186072	Cat	Tekes *et al.*, 2008
Feline coronavirus type II	AY994055	Cat	Barker *et al.*, 2013
Porcine respiratory coronavirus	DQ811787	Pig	Zhang *et al.*, 2007b
Transmissible gastroenteritis virus	AJ271965	Pig	Almazan *et al.*, 2000
Human coronavirus 229E	NC_002645	Human	Thiel *et al.*, 2001
Human coronavirus NL63	NC_005831	Human	van der Hoek *et al.*, 2004
Miniopterus bat coronavirus 1			
Miniopterus bat coronavirus 1A	NC_010437	Bat	Chu *et al.*, 2008
Miniopterus bat coronavirus 1B	NC_010436	Bat	Chu *et al.*, 2008
Miniopterus bat coronavirus HKU8	NC_010438	Bat	Chu *et al.*, 2008
Porcine epidemic diarrhea virus	NC_003436	Pig	Bridgen *et al.*, 1998
Rhinolophus bat coronavirus HKU2	NC_009988	Bat	Lau *et al.*, 2007
Scotophilus bat coronavirus 512	NC_009657	Bat	Tang *et al.*, 2006
Genus *Betacoronavirus*			
Betacoronavirus 1			
Bovine coronavirus	NC_003045	Cattle	Chouljenko *et al.*, 2001
Canine respiratory coronavirus	JX860640	Dog	Lim *et al.*, 2013
Equine coronavirus	NC_010327	Horse	Zhang *et al.*, 2007a
Humancoronavirus OC43	NC_005147	Human	Vijgen *et al.*, 2005
Dromedary camel coronavirus HKU23	KF906251	Camel	Woo *et al.*, 2014
Murine coronavirus			
Murine hepatitis virus	NC_001846	Mouse	Leparc-Goffart *et al.*, 1997
Rat coronavirus	NC_012936	Rat	Stephensen *et al.*, 1999
Human coronavirus HKU1	NC_006577	Human	Woo *et al.*, 2005
Pipistrellus bat coronavirus HKU5	NC_009020	Bat	Woo *et al.*, 2007
Rousettus bat coronavirus HKU9	NC_009021	Bat	Woo *et al.*, 2007
Tylonycteris bat coronavirus HKU4	NC_009019	Bat	Woo *et al.*, 2007
Severe acute respiratory syndrome-related coronavirus			
SARS-related human coronavirus	NC_004718	Human	Marra *et al.*, 2003
SARS-related palm civet coronavirusSZ3	AY304486	Civet	Guan *et al.*, 2003
SARS-related Rhinolophus bat coronavirus Rp3	DQ412042	Bat	Li W *et al.*, 2005a

(*Continued*)

TABLE 5.2 (*Continued*)

Species	GenBank accession No. of representative strains	Host	Reference
Genus *Deltacoronavirus*			
Bulbul coronavirus HKU11	FJ376620	Avian	Woo *et al.*, 2009
Thrush coronavirus HKU12	NC_011549	Avian	Woo *et al.*, 2009
Munia coronavirus HKU13	NC_011550	Avian	Woo *et al.*, 2009
Genus *Gammacoronavirus*			
Avian coronavirus			
Duck coronavirus	JF705860	Avian	Chen *et al.*, 2013
Infectious bronchitis virus	AY646283	Avian	Binns *et al.*, 1986
Turkey coronavirus	EU022526	Avian	Cao *et al.*, 2008
Beluga Whale coronavirus SW1	NC_010646	Whale	Mihindukulasuriya *et al.*, 2008

The overall genomic organization of SARS-CoV is similar to that of other coronaviruses. Six open reading frames (ORFs) are conserved throughout the *Coronavirnae* and arranged in the 5´ to 3´ direction: ORFs 1a and 1b, together comprising the *replicase* genes and taking approximately two-thirds of the genome, and the ORFs encoding for the structural proteins including the spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N). Between ORF1b and the structural protein genes, or within the N gene, there are eight auxiliary genes: ORF3a, 3b, 6, 7a, 7b, 8a, 8b, and 9b which are specific to SARS-CoV (Chim *et al.*, 2003; Rota *et al.*, 2003; Ruan *et al.*, 2003; Snijder *et al.*, 2003) (Figure 5.1). By phylogenetic analysis using the conserved polymerase genes, SARS-CoV forms a distinct group within the genus *Betacoronavirus*. These distinct genomic characteristics classify SARS-CoV as a novel coronavirus species (Gorbalenya *et al.*, 2004).

5.2.1.2 Animal origins of SARS-CoV Epidemiological studies indicated that all initial SARS cases had recently had contact with animals. The search for the animal origin of SARS-CoV was first conducted in 2003 in a live animal market in Shenzhen, Guangdong Province. Serological evidence of SARS-CoV was discovered in masked palm civets (*Paguma larvata*), raccoon dogs (*Nyctereutes procyonoides*), and Chinese ferret badgers (*Melogale moschata*) (Guan *et al.*, 2003). Two full-length genomic sequences, SZ3 and SZ16, were identified from nasal swabs from masked palm civets. The genomes of SZ3 and SZ16 showed 99.8% nucleotide (nt) pairwise identities to that of human SARS-CoV strain Tor2. But a 29 nt insertion was found in ORF8 in the genome of SZ3 and SZ16, which forms a complete ORF8 instead of two split ORFs as in the human SARS-CoV. This insertion was also found in a few of the early human SARS isolates, but was completely deleted in most of the isolates of the early phase patients and all isolates of the middle and late phase patients. This breakthrough initially connected SARS-CoV to the masked palm civet (Guan *et al.*, 2003).

In succession, different research teams investigated the antibodies of SARS-CoV in both wild and domestic animal traders, market managers, and food traders in markets in

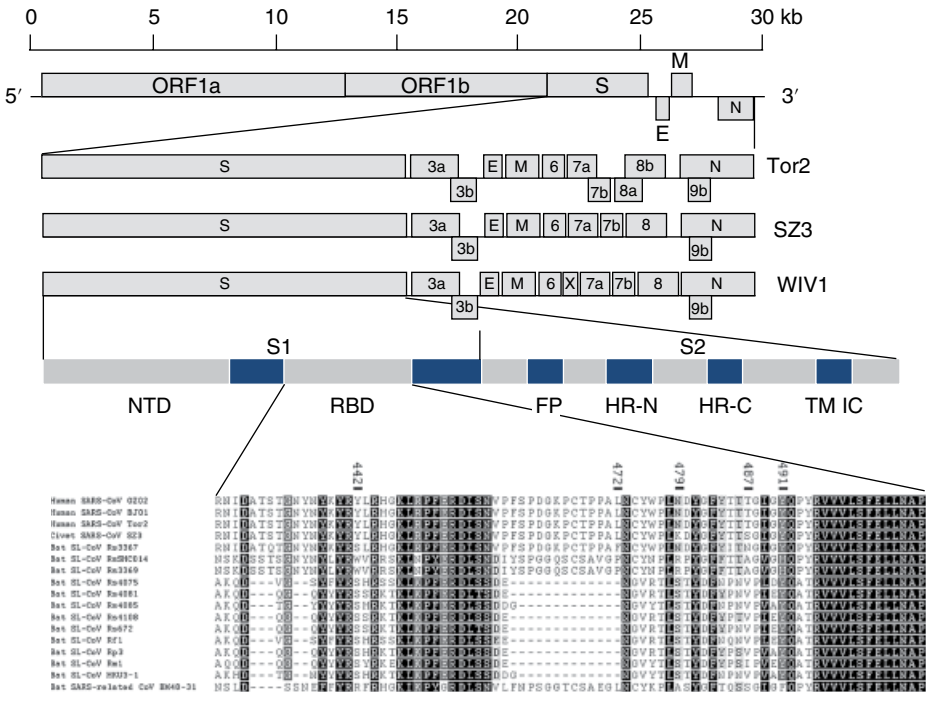

Figure 5.1 Comparison of genome organization and RBD sequences of SARS-CoVs and SL-CoVs, Genes are identified by their transcript names (1a, 1b, 3a, 3b, 6, X, 7, 7a, 7b, 8, and 9b), or the abbreviated name of their protein product (S, spike; E, envelope; M, membrane; N, nucleocapsid). Partial sequences (426–507 aa) of the RBD domain containing the key receptor binding sites (indicated by amino acid numbers) are shown. NTD, N-terminal domain; RBD, receptor binding domain; HR-N, heptad-repeat N-terminal domain; HR-C, heptad-repeat C-terminal domain; TM, transmembrane domain; IC, intracellular tail. Literature references are described in the text. GenBank accession numbers: WIV1 (bat SARS-like CoV WIV1), KF367457; Tor2 (human SARS-CoV Tor2), JX163924; SZ3 (civet SARS-CoV SZ3), AY304486; Rs3367, KC881006; RsSCH014, KC881005; Rs3369, KC880986; Rs4075, KC880993; Rs4081, KC880999; Rs4085, KC880992; Rs4108, KC881001; Rp3, DQ071615; Rs672, FJ588686; Rf1, DQ412042; Rm1, DQ412043; HKU3, DQ022305; BM48–31, NC_014470.

Shenzhen and Guangzhou. The data showed that the prevalence of antibodies in traders who dealt with masked palm civets could be as high as 72.7%, which was significantly higher than that in other human populations (Guan *et al.*, 2003; Xu *et al.*, 2004a; Xu *et al.*, 2004b). These results suggested that SARS-CoV infection was due to direct exposure to wild animals (especially masked palm civets) (Xu *et al.*, 2004a, b).

During the second stage of the SARS outbreak from 16 December 2012 to 8 January 2013, four patients in Guangzhou were diagnosed with SARS-CoV infection (Liang *et al.*, 2004). All four patients had had direct or indirect contact with wild animals or house rats before the onset of clinical symptoms. Consistently, SARS-CoV was detected by reverse transcription–polymerase chain reaction (RT-PCR) in palm civets and raccoon dogs from markets and restaurants in Guangzhou. The full-length genomic

sequences of SARS-CoV from two patients and the palm civets had 99.89% similarity (Chinese SARS Molecular Epidemiology Consortium, 2004; Kan *et al.*, 2005; Song *et al.*, 2005). These results again suggested that civets were the origin of human SARS-CoV during the 2003–2004 outbreak.

To further confirm civets as natural or reservoir hosts of SARS-CoV, Kan *et al.* (2005) investigated >1000 farmed civets in 12 Provinces in China in 2004. In contrast with the market civets in Guangdong Province during the SARS outbreak, all of the farmed civets tested negative for SARS-CoV by RT-PCR assay (Kan *et al.*, 2005). An investigation conducted in wild civets collected in Hong Kong confirmed that they were not infected by SARS-CoV (Poon *et al.*, 2005). At the same time, Wu *et al.* (2005) performed an experimental infection in civets using two human isolates of SARS-CoV, BJ01 (with the 29 nt deletion) and GZ01 (without the 29 nt deletion). All 10 inoculated civets displayed clinical symptoms such as fever, lethargy, and loss of aggressiveness (Tu *et al.*, 2004; Wu *et al.*, 2005). This demonstrated that civets were just as susceptible to SARS as humans. These findings suggested that civets may not be natural reservoir hosts of SARS-CoV after all, but rather were intermediate hosts that had facilitated transmission from its natural reservoir into humans.

5.2.1.3 Bat SARS-like CoV In 2005 two independent teams in China reported their discovery of coronaviruses closely related to human SARS-CoV in horseshoe bats. Both teams found serological and genomic evidence of SARS-like coronavirus (SL-CoV) in bat samples collected from Guangdong, Guangxi, Hubei, Tianjin, and Hong Kong. All bat SL-CoV positive samples came from *Rhinolophus* bats in the family Rhinolophidae, including *R. sinicus*, *R. pusillus*, *R. macrotis*, and *R. ferrumequinum* (Lau *et al.*, 2005; Li W *et al.*, 2005a; Ren *et al.*, 2006; Yuan *et al.*, 2010). Full-length genomic sequences of four bat SL-CoV isolates (HKU3, Rp3, Rf1, and Rm1) were identified from bat fecal samples. The genomic sequence comparison revealed that these bat SL-CoVs shared identical genomic organization and had 87–92% nt identities to human or civet SARS-CoVs. Like the coronaviruses in civets or early phase patients, bat SL-CoVs were shown to have a 29 nt insertion in ORF8. Except for the spike protein, ORF3, and ORF8, all bat SL-CoV proteins shared high amino acid sequence identities of 93–100% with their homologs in human or civet SARS-CoVs (Lau *et al.*, 2005; Li W *et al.*, 2005a; Ren *et al.*, 2006).

Subsequent investigations demonstrated that SL-CoVs were not only present throughout China, but also in Europe and Africa (Drexler *et al.*, 2010; Lau *et al.*, 2010a; Yuan *et al.*, 2010). In Europe, a high prevalence of SL-CoVs was detected in *Rhinolophus* bat species in Bulgaria, Slovenia, and Italy. These European SL-CoVs were genetically distinct from those from China. The Slovenian strains shared 85% nucleotide identity and 95.6% amino acid identity to the Chinese strain Rp3, while the Bulgarian strain BM48–31 was shown to be highly divergent from Chinese SL-CoVs in proteins encoded by ORF3b and ORF6, and lack the coding capacity for ORF8 (Drexler *et al.*, 2010; Rihtaric *et al.*, 2010; Balboni *et al.*, 2011). In Africa, betacoronaviruses related to SARS-CoV were detected in both *Hipposideros* and *Chaerephon* bats from Ghana, Kenya, and Nigeria. Compared to *Rhinolphus* SL-CoVs from Eurasia, the betacoronaviruses from African non-*Rhinolophus* bat species were much more phylogenetically distant to SARS-CoV. For example, Zaria bat coronavirus from *Hipposideros commersoni* in Nigeria possesses three overlapping ORFs between the M and N genes and two conserved stem-loop II motifs (Pfefferle *et al.*, 2009; Tong *et al.*, 2009; Quan *et al.*, 2010).

Prior to 2013, all discoveries of SL-CoVs were based on genomic evidence. No SL-CoV had been successfully isolated *in vitro*. In 2013 a breakthrough was achieved that provided the strongest evidence to date of the origin of SARS-CoV. At a single bat colony in Yunnan Province, Ge *et al*. (2013) conducted a 12-month longitudinal survey (April 2011–September 2012) of SL-CoVs in a colony of *Rhinolophus sinicus* bats. Both a high prevalence and high genetic diversity of SL-CoVs were observed. Analysis of the S protein sequences indicated the presence of seven different strains of SL-CoVs existing in the bat colony including two newly detected strains and five other strains similar to Rs672, HKU3–1, Rp3, Rf1, and Rm1, respectively (Ge *et al.*, 2013). The full-length genomes of the two novel strains (SL-CoV RsSHC014 and Rs3367) were determined by sequencing. The overall nucleotide sequence identity between SL-CoV RsSHC014, Rs3367 and human SARS-CoV genomes (Tor2 strain) was 95%, much higher than previously observed for bat SL-CoVs in China (88–92%) or Europe (76%). Higher sequence identities were also observed at the protein level between these new SL-CoVs and SARS-CoVs, particularly on the S proteins in which no deletions were observed (Figure 5.1). Most importantly, a live SL-CoV (SL-CoV WIV1) was isolated from bat fecal samples. Furthermore, SL-CoV WIV1 was demonstrated to use the same receptor as the SARS-CoV for cell entry. Serum-neutralization assays, using nine convalescent sera from human SARS patients, showed that seven of these were able to completely neutralize 100 $TCID_{50}$ (tissue culture infectious dose 50) of WIV1 at dilutions between 1:10 to 1:40, further confirming the close relationship between WIV1 and SARS-CoV. These data provided the strongest evidence to date that Chinese horseshoe bats are the natural reservoir of SARS-CoV.

5.2.1.4 Mechanisms of interspecies transmission of SARS-CoV Understanding the mechanism of how SARS-CoV was transmitted to humans is a big concern. The S protein of coronaviruses is responsible for receptor binding, fusion, and determining viral host tropism (Lai *et al.*, 2007). The S protein is a membrane-bound trimer and contains two subunits; receptor-binding subunit S1 and membrane-fusion subunit S2 (Figure 5.1). The S2 subunits from *Alphacoronvirus* and *Betacoronavirus* share sequence and structural homology and also contain homologous heptad-repeat segments that fold into a conserved trimer-of-hairpins structure essential for membrane fusion (Zheng *et al.*, 2006). The S1 subunits from *Alphacoronvirus* and *Betacoronavirus* have no obvious sequence homology. The S1 regions contain receptor-binding domains (RBD) that are sufficient for high-affinity binding to a viral host receptor (Gallagher & Buchmeier, 2001).

A metallopeptidase, angiotensin-converting enzyme 2 (ACE2) was identified as the functional receptor of the SARS-CoV (Li *et al.*, 2003). Detailed peptide mapping revealed that a fragment of 193 aa (aa 318–510) in the S protein was sufficient to bind human ACE2 (Wong *et al.*, 2004).

Based on the epidemiologic data, Li *et al*. (2005b) investigated the molecular mechanism of interspecies transmission of SARS-CoV from non-human animals to humans by using two human SARS-CoV strains isolated from the 2002–2003 (Tor2) and 2003–2004 (GZ03) SARS outbreaks, and one strain isolated from palm civets (SZ3). They found that all three S proteins bound to and utilized palm civet ACE2 efficiently, but GZ03 and SZ3 S proteins utilized human ACE2 markedly less efficiently than did the Tor2 S protein. Binding assays combining the various point mutations indicated that the

difference in binding efficiency was caused by the alteration of S protein residues 479 and 487, and the adaptation of the S protein to human ACE2 is facilitated by alteration of residue 479 to asparagine and of 487 to threonine (Li W *et al.*, 2005b; Qu *et al.*, 2005). In the second SARS outbreak (2003–2004), the individuals infected by GZ03 appeared to have less severe symptoms and no secondary transmission was observed, all of which may have been due to fewer mutations of key residues in the S proteins of GZ03 (Li W *et al.*, 2005b). The structure of SARS-CoV S protein RBD (aa 306–527) complexed with human receptor ACE2 (aa 19–615) further confirmed the above results (Li F *et al.*, 2005). In addition, Li (2008) resolved the complexed structures of RBDs from various human and civet SARS-CoV strains with a chimeric ACE2 bearing the critical N-terminal helix from civet and the remaining peptidase domain from human. The results showed that the major species barriers are determined by interactions between four ACE2 residues (residues 31, 35, 38, and 353) and two RBD residues (residues 479 and 487) (Li, 2008).

For bat SL-CoVs, Ren *et al.* (2008) analyzed the receptor usage of one SL-CoV strain (Rp3) by combining a human immunodeficiency virus-based pseudovirus system with cell lines expressing ACE2 molecules of humans, civets, or horseshoe bats (Ren *et al.*, 2008). The results demonstrated that the previously discovered SL-CoV strain Rp3 could not use ACE2 as receptor. However, the chimeric Rp3-S protein with a minimal insert region (human SARS-CoV S amino acids 310–518) gained the ability to enter cells via human ACE2 (Ren *et al.*, 2008). This result was further confirmed by a recombinant bat SL-CoV based on the HKU3 genome backbone carrying the RBD of the human SARS-CoV S in ACE2 humanized mice (Becker *et al.*, 2008). These results indicate that very few evolutionary changes (likely recombination events) may be required to confer bat SL-CoV with the ability to infect humans.

Receptor analysis of the recent SL-CoV isolate WIV1 revealed another possible route of coronavirus transmission to humans (Ge *et al*., 2013). It was demonstrated that WIV1 replicates efficiently in HeLa cells expressing ACE2 from humans, civets, or Chinese horseshoe bats and that it can grow in African green monkey kidney cells (Vero E6), human alveolar basal epithelial cells (A549), pig kidney 15 cells (PK-15), and *Rhinolophus sinicus* kidney cells (RSKT). WIV1 has high similarity in the RBD (96% identity) with human SARS-CoV. Of the two critical residues described above, residue 479 is identical (asparagine) between WIV1 and human SARS-CoV. Instead of threonine at residue 487 for SARS-CoV, WIV1 has asparagine. The ability of SL-CoV WIV1 to use the human ACE2 receptor suggests that direct bat to human infection is a plausible scenario for some bat SL-CoVs.

5.2.2 Middle East respiratory syndrome (MERS)

5.2.2.1 MERS and MERS-CoV The first human MERS case was reported on 13 June 2012 in Jeddah, Saudi Arabia. Shortly thereafter a novel coronavirus HCoV-EMC/2012 now known as MERS-CoV was isolated from a patient (Bermingham *et al.*, 2012; Zaki *et al.*, 2012; de Groot *et al.*, 2013). By 23 February 2015 a total of 1026 laboratory-confirmed cases of MERS-CoV infection, including at least 376 related deaths (WHO 2015) have been reported – about 37% mortality. Since its first documented case in Saudi Arabia, MERS-CoV infection has been reported throughout the Middle East: United Arab Emirates, Qatar, Oman, Jordan, Kuwait, Yemen, Lebanon, and Iran. Spread by travellers, the infection was reported globally in the United

Kingdom, France, Tunisia, Italy, Malaysia, Philippines, Greece, Egypt, United States, Netherlands, and Algeria. Current epidemiological data show that MERS-CoV exhibits limited human-to-human transmission (Health Protection Agency UK Novel Coronavirus Investigation team, 2013; Mailles *et al.*, 2013). Phylogenetic analysis of the *replicase* gene of coronaviruses with completely sequenced genomes showed that MERS-CoV is most closely related to two Chinese bat coronavirus species in the genus *Betacoronavirus*: *Tylonycteris bat coronavirus HKU4* (BtCoV-HKU4) and *Pipistrellus bat coronavirus HKU5* (BtCoV-HKU5) (Woo *et al.*, 2007). MERS-CoV was found to have 75% and 77% amino acid sequence identity, respectively, with BtCoV-HKU4 and BtCoV-HKU5, based on conserved *replicase* genes. According to the classification criteria of the ICTV, MERS-CoV represents a novel coronavirus species (van Boheemen *et al.*, 2012). Sequence comparison revealed that similar viral sequences to MERS-CoV have been detected in *Pipistrellus pipistrellus* in the Netherlands in 2008 and *Eptesicus isabellinus* in Spain in 2011 prior to the outbreak of MERS (Falcon *et al.*, 2011; Reusken *et al.*, 2010). MERS-CoV, HKU4, HKU5, and several similar coronaviruses from Europe cluster together as a new group within the *Betacoronavirus* genus.

Based on full-length genomic sequences, MERS-CoVs isolated from patients are phylogenetically classified into two lineages: A and B. The viral genomes detected in the earliest cases in humans (MERS-CoV and Jordan-N3/2012) fall into lineage A and are genetically distinct from the later cases (England-Qatar/2012, England2-HPA, and others) that fall into lineage B. The accumulation of genetic diversity, including changes in the S protein, suggests that the natural reservoirs of MERS-CoV are geographically widespread (Cotten *et al.*, 2013).

5.2.2.2 Animal origins of MERS-CoV Because of the phylogenetic similarities between MERS-CoV and bat coronaviruses, the search for the animal reservoir of MERS-CoV was initially focused on bats. A betacoronavirus closely related to MERS-CoV was detected in *Nycteris* bats in Ghana with a prevalence of 24.9% and clustered as a basal sister clade with MERS-CoV, HKU4, and HKU5. *Pipistrellus* bats in Romania and Ukraine were found to harbor coronaviruses which share higher homologs with MERS-CoV than HKU4 and HKU5, around 87.1 to 88.1% nucleotide identity and 98.3% amino acid identity based on a 904-bp fragment of the RdRp gene (Annan *et al.*, 2013). Another betacoronavirus was reported in *Eptesicus serotinus* from northern Italy and this strain (ITA26/384/2012) shared 96.9% amino acid identity with MERS-CoV in an 816-bp RdRp fragment (De Benedictis *et al.*, 2014). In the Western Hemisphere a MERS-related coronavirus was discovered in a *Nyctinomops laticaudatus* bat from Mexico. Its partial non-structural protein 14 (nsp14) sequence had 85.7% nucleotide identity and 95.5% amino acid identity with that of MERS-CoV (Anthony *et al.*, 2013b). Nevertheless, none of the bat betacoronaviruses mentioned above is likely to be a direct ancestor of MERS-CoV (Lau *et al.*, 2013).

Available epidemiology data indicate that some MERS patients had a history of exposure to dromedary camels and goats (Albarrak *et al.*, 2012; Buchholz *et al.*, 2013). Serological evidence against the MERS-CoV S protein revealed that Omani camels – but not European sheep, goats, cattle, and other camelids – had a high prevalence of neutralizing antibodies against MERS-CoV (Hemida *et al.*, 2013; Reusken *et al.*, 2013a, b; Alagaili *et al.*, 2014; Alexandersen *et al.*, 2014). A retrospective search for MERS-CoV antibodies indicated that the virus could be traced to as early as 1992 in the Middle East (Alagaili *et al.*, 2014; Alexandersen *et al.*, 2014; Meyer *et al.*, 2014).

Genomic sequences and viral isolation further confirmed the origin of MERS-CoV in Camels. Haagmans *et al.* (2014) first detected MERS-CoV RNA from 3 out of 14 dromedary camels from a farm in Qatar linked to two human cases in October 2013 (Haagmans *et al.*, 2014). Chu *et al.* (2014) identified MERS-CoV from nasal swab specimens in 4 out of 110 apparently healthy dromedaries in Egypt and obtained a near-full-length genome sequence of camel MERS-CoV (NRCE-HKU205), which had an overall nucleotide similarity of 99.2–99.5% to the human isolate. Unlike human MERS-CoVs, NRCE-HKU205 has 12 aa differences (residues 23, 26, 194, 434, 666, 696, 756, 886, 888, 918, 1158, and 1333) in the S protein (Chu *et al.*, 2014). In June 2014 direct evidence was provided for camel-to-human transmission of MERS-CoV when a previously healthy 43-year-old Saudi man developed respiratory symptoms after caring for ill camels, several of which had been exhibiting nasal discharge (Azhar *et al.*, 2014; Memish *et al.*, 2014). MERS-CoVs isolated from the nasal swabs of this patient and from one of the camels were almost identical (Azhar *et al.*, 2014). Phylogenetic analysis of MERS-CoV genomes obtained from human cases and camels suggests that multiple zoonotic spill-over events have occurred since the beginning of the MERS-CoV epidemic (Cotten *et al.*, 2013, 2014; Alagaili *et al.*, 2014; Briese *et al.*, 2014; Kupferschmidt, 2014). These data further suggest that MERS-CoVs have been circulating in dromedary camels for at least two decades if not longer and can be transmitted from camels to humans through close contact.

5.2.2.3 Mechanisms of interspecies transmission of MERS-CoV Viral receptor analysis is important in understanding the interspecies transmission of MERS-CoV and is helpful for antiviral drug screening and vaccine development. Soon after the MERS outbreak, a cellular molecule, dipeptidyl peptidase 4 (DPP4, also known as CD26) was identified as a functional receptor for MERS-CoV (Raj *et al.*, 2013). DPP4 is relatively conserved among mammalian species, and most cell lines derived from human, bat, non-human primate or swine were found to be susceptible to MERS-CoV infection. However, cell lines originating from mice, hamsters, dogs, and cats were not susceptible (Chan *et al.*, 2013; Raj *et al.*, 2013). DPP4 from camel, goat, cow, and sheep can be also recognized by MERS-CoV and can support MERS-CoV replication (Barlan *et al.*, 2014; van Doremalen *et al.*, 2014). These findings suggest that the MERS-CoV receptor DPP4 plays a pivotal role in the observed species tropism of MERS-CoV and may be a restriction factor for interspecies transmission of MERS-CoV.

5.3 GENETIC DIVERSITY OF BAT CORONAVIRUSES

5.3.1 Alphacoronaviruses

Alphacoronaviruses infect various mammalian species including humans, pigs, cats, and bats (Pedersen *et al.*, 1984; Kusanagi *et al.*, 1992; van der Hoek *et al.*, 2004; Chu *et al.*, 2008). Among the eight currently established species within the genus *Alphacoronavirus*, four were identified in Chinese insectivorous bats: *Miniopterus bat coronavirus 1* (1A and 1B), *Miniopterus bat coronavirus HKU8*, *Scotophilus bat coronavirus 512*, and *Rhinolophus bat coronavirus HKU2* (Tang *et al.*, 2006; Lau *et al.*, 2007; Chu *et al.*, 2008) (Table 5.1 and Figure 5.2).

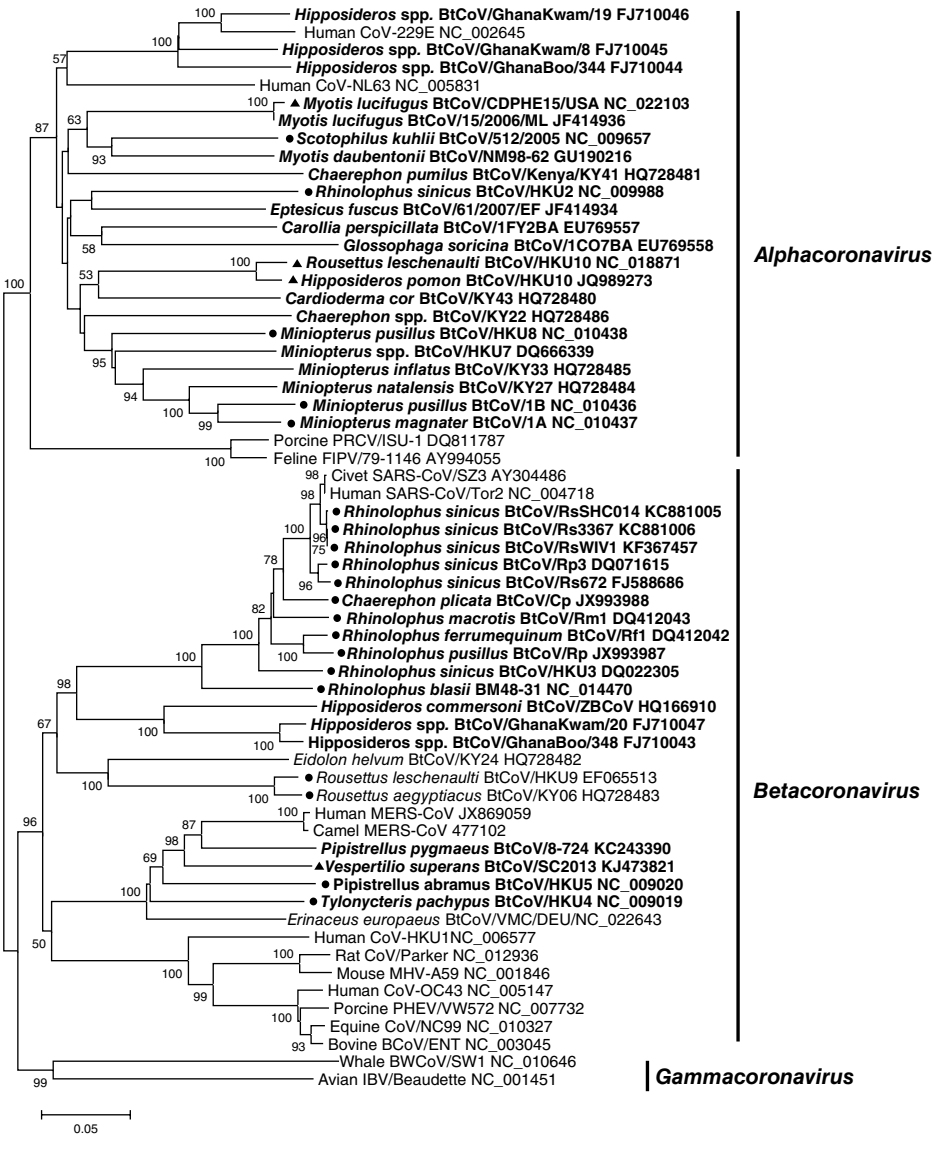

Figure 5.2 Phylogenetic analysis of coronaviruses derived from bats and other species. The phylogenetic tree was constructed based on partial RdRp sequences (816 nt). The available RdRp sequences (≥816 nt) were extracted from GenBank and aligned using ClustalW. The alignment was used for tree construction by the neighbor-joining method using MEGA (Version 5.1). Numbers above branches indicate bootstrap values calculated from 1000 bootstrap replicates (values ≥50 are shown). All the coronaviruses derived from bats are drawn in bold. The bat coronaviruses were named following bat species, plus BtCoV, strain name, and GenBank accession number. Classified bat coronavirus species are indicated by filled circles. Unclassified bat coronaviruses with full-length genomes are indicated by filled triangles.

The first bat alphacoronavirus, designated *Miniopterus bat coronavirus 1* (BtCoV 1), was reported from three different *Miniopterus* bat species in Hong Kong (Poon *et al.*, 2005). The high prevalence rate (63%) of this virus in *Miniopterus pusillus* suggests that it might be a commonly circulating coronavirus in this species in Hong Kong. In two subsequent studies targeting bats in Hong Kong, four distinct alphacoronaviruses including BtCoV 1A, BtCoV 1B, BtCoV HKU7, and BtCoV HKU8 were found in *Miniopterus* bats. These viruses are closely related genetically and are derived from a common ancestor (Chu *et al.*, 2006; Woo *et al.*, 2006). BtCoV 1A and 1B are two very close – but distinct – lineages divided from the previously reported BtCoV 1 and they have apparent host restriction to *Miniopterus magnater* and *M. pusillus*, respectively. Moreover, coinfections of BtCoV 1B and HKU8 were commonly observed among *M. pusillus* (Chu *et al.*, 2008). With the availability of full genome sequences, BtCoV 1A and 1B have been assigned to the same species within the genus *Alphacoronavirus*, known as *Miniopterus bat coronavirus 1*. BtCoV HKU8 represents another species. The co-presence of genetically diverse but related alphacoronaviruses in *Miniopterus* bats in a small geographical region suggests that alphacoronaviruses have coevolved in this genus for a long time (Chu *et al.*, 2006).

Besides *Miniopterus*, bats of other genera in China have also been demonstrated to harbor alphacoronaviruses. Genetically divergent alphacoronaviruses were found in *Myotis*, *Scotophilus*, and *Rhinolophus* bats from different locations in China. Phylogenetic analysis has revealed host species specificity among these bat coronaviruses (Tang *et al.*, 2006; Woo *et al.*, 2006; He *et al.*, 2014). Interestingly, an alphacoronavirus identified in *Rhinolophus sinicus* from Hong Kong (BtCoV HKU2) possesses a unique genetic feature compared to all other alphacoronaviruses; its spike protein contains a short peptide homologous to a corresponding peptide within the RBD of SARS-CoV S protein. This suggests that the spike protein of BtCoV HKU2 could have been acquired from SARS-CoV via recombination (Lau *et al.*, 2007). Another alphacoronavirus (BtCoV HKU10) was detected in insectivorous bats (*Hipposideros pomona*) from Hong Kong and fruigivorus bats (*Rousettus leschenaultii*) from Guangdong and potentially represents a novel alphacoronavirus species. The genome sequences of *Hipposideros* CoV HKU10 and *Rousettus* CoV HKU10 share high similarity except in the S gene. Evidence was found for a recent transmission of BtCoV HKU10 from *R. leschenaultii* to *H. pomona* and it is the first evidence for interspecies transmission of coronavirus between different suborders of bats (Lau *et al.*, 2012)

In addition to China, detection of alphacoronaviruses in bats has been reported in many other countries throughout the globe. The coronaviruses detected in *Miniopterus fuliginosus* from Japan show a close relationship to BtCoV HKU7 from *M. magnater* in Hong Kong (Shirato *et al.*, 2012). In the Philippines two alphacoronaviruses were found in *Scotophilus khulii* and *Hipposideros diadema*, respectively, and they share the highest nucleotide sequence identity of 95% and 80% respectively to the strains previously described in China in the partial RdRp gene (Tsuda *et al.*, 2012; Watanabe *et al.*, 2010). In Europe a number of alphacoronaviruses with a wide diversity and distribution were reported from multiple bat species in Spain and Germany, including *Myotis* sp., *Pipistrellus* sp., and *Nyctalus lasiopterus*. Some European bat alphacoronaviruses are related to those found in Asia while others are distinct and clustered in new monophyletic clades (Gloza-Rausch *et al.*, 2008; Drexler *et al.*, 2011; Falcon *et al.*, 2011). A great diversity of bat alphacoronavirus is also present in Africa. Three different coronaviruses

BtKY22, BtKY41, and BtKY43 were identified from *Chaerephon* and *Cardioderma* bats in Kenya. Genomic characterization suggests they are members of the genus *Alphacoronavirus*, but they are phylogenetically distant from any other bat coronaviruses and likely to represent three novel species. Additionally, viruses belonging to the established species *Miniopterus bat coronavirus 1* were detected in Kenyan bats as well (Tao *et al.*, 2012). In North America where bat species different from the Eastern Hemisphere are distributed, three clusters of alphacoronaviruses were found in *Eptesicus fuscus* and *Myotis occultus* inhabiting the Rocky Mountain region and exhibit significant dissimilarity with the alphacoronaviruses of Asian bats in the highly conserved RdRp region (Dominguez *et al.*, 2007). More recently a novel alphacoronavirus was discovered in guano of lesser short tailed bats (*Mystacina tuberculata*) on a remote offshore island in New Zealand with 80% nucleotide identity to BtCoV HKU8. Interestingly, despite the geographic and evolutionary isolation of the host species, this virus has not diverged significantly from other alphacoronaviruses (Hall *et al.*, 2014). Moreover, although most studies suggest host species restriction of bat alphacoronavirus, different bat species from the same colony have been found to harbor alphacoronaviruses of the same genetic lineage, which indicates a great complexity of the ecology of this viral genus in bats (Tang *et al.*, 2006; Falcon *et al.*, 2011).

Finally, there have been two reports of bat alphacoronaviruses closely related to human pathogenic coronaviruses. BtCoV Hipposideros/GhanaKwam/19/2008 was detected in *Hipposideros caffer ruber* in Ghana. Its RdRp fragment shares 92% nucleotide sequence identity with Human coronavirus 229E and they are predicted to share a most recent common ancestor only 200 years ago (Pfefferle *et al.*, 2009). Another bat coronavirus derived from the North American tricolored bat (*Perimyotis subflavus*) was predicted to share common ancestry with Human coronavirus strain NL63. Their most recent common ancestor was calculated to have occurred approximately 563–822 years ago (Huynh *et al.*, 2012).

In summary, alphacoronaviruses infect a wide range of different species and exhibit remarkably high genetic diversity in bats. Natural infection of different bat species with different alphacoronaviruses is globally present and bats are suggested to be an ancestral source of this coronavirus genus. More alphacoronaviruses have yet to be discovered in bats elsewhere in the near future.

5.3.2 Betacoronaviruses

Compared with bat alphacoronaviruses, bat betacoronaviruses have been identified from fewer host species and show less genetic diversity (He *et al.*, 2014). Bat betacoronaviruses are distributed among three of the four betacoronavirus lineages. *Betacoronavirus* group B contains diverse SARS-like bat coronaviruses while group C betacoronaviruses include diverse MERS-related bat coronaviruses. These viruses have already been discussed above.

The other bat-associated betacoronavirus species, *Rousettus bat coronavirus HKU9*, is currently the sole species belonging to *Betacoronavirus* group D. BtCoV HKU9 was first discovered in *R. leschenaultii* bats in Guangdong Province in China. Complete genome sequencing of four BtHKU9 strains revealed a marked nucleotide and amino acid sequence polymorphism among isolates (Woo *et al.*, 2012). Interestingly, the same bat could be coinfected with two or three distinct genotypes of

BtCoV HKU9. The presence of diverse genotypes of BtCoV HKU9 in *R. leschenaultii* bats is likely due to a combination of mutation and recombination that may have been facilitated by dense roosting behavior and long range foraging of this particular bat species (Lau *et al.*, 2010b). Additionally, BtCoV HKU9 strains were detected in *Hipposideros* sp. samples collected in Yunnan Province (Ge *et al.*, 2012). The sequence variation between different strains is consistent with what was found in *R. leschenaultii* in Guangdong and further demonstrates the genetic diversity of BtCoV HKU9 in bat populations.

Although they are not as abundant or diverse as bat alphacoronaviruses, studies of the distribution, genetic diversity, and evolution of bat betacoronaviruses are of special importance, since many pose potential threats to human health. It is highly likely that additional betacoronaviruses will be identified in bats. The huge diversity of alphacoronaviruses and betacoronaviruses in bats supports the hypothesis that bats are ideal hosts for these viruses and fuel the evolution and dissemination of these two genera (Woo *et al.*, 2012).

5.3.3 Gammacoronaviruses

Currently, the sole recorded bat gamacoronavirus (PgCoV-4) was found in one Indian bat (*Pteropus giganteus*) in Bangladesh (Anthony *et al.*, 2013a). The sequence of a partial RdRp fragment (294 nt) of PgCoV-4 is close to avian infectious bronchitis virus (IBV) with 92% nt identity and 98% aa identity, respectively. Phylogenetically, PgCoV-4 closely clusters with IBV and falls into the genus *Gammacoronavirus*.

5.3.4 Classification of coronaviruses

According to the ICTV criteria for classification of coronaviruses, only viruses with complete genome sequences are considered for taxonomy. This standard compares the pair-wise evolutionary distances using coronavirus family-wide conserved domains in the replicase polyprotein pp1ab, which consists of seven peptide subunits: nsp3, nsp5, nsp12 (RdRp), nsp13, nsp14, nsp15, and nsp16. Viruses sharing less than 90% sequence identity in the conserved replicase domains with any other established member of the family may be considered representatives of a new species. Viruses sharing less than 46% sequence identity in the aforementioned conserved replicase domains with any other established members of the family may be considered representatives of a new genus (de Groot *et al.*, 2012). Under these criteria, 59 bat coronaviruses with full-length genome sequences were divided into eight established species, including *Miniopterus bat coronavirus 1* (2 genomes, 1A and 1B), *Rhinolophus bat coronavirus HKU2* (4 genomes), *Miniopterus bat coronavirus HKU8* (one genome), *Scotophilus bat coronavirus 512* (one genome), *Tylonycteris bat coronavirus HKU4* (5 genomes), *Pipistrellus bat coronavirus HKU5* (4 genomes), *Rousettus bat coronavirus HKU9* (8 genomes), and *Severe acute respiratory syndrome-related coronavirus* (25 genomes, HKU3, Rp3, WIV1, and etc), and 2 unassigned species including *Bat coronavirus HKU10* (8 genomes) and *Bat coronavirus CDPHE15/USA/2006* (one genome) (Table 5.1). For investigation of bat coronaviruses, most research is based on PCR assays targeting the conserved RdRp fragment. Usually, the PCR amplicon sizes range from 121 nt to 440 nt

(Tong *et al.*, 2009; Anthony *et al.*, 2013b; Memish *et al.*, 2013). Based on these RdRp fragments, these viruses may be roughly assigned to a new species or a new genus. Complementary to this, Drexler *et al*. (2010) developed RdRp-based grouping units (RGU) for coronaviruses by comparing amino acid identities translated from an 816-bp fragment of the RdRp. Criteria for defining separate RGU in mammalian CoV were greater than 4.8% amino acid distance for alphacoronaviruses and greater than 6.3% distance for betacoronaviruses. Recently, human MERS-CoV and *Pipistrellus* bat HKU5 were found to have only 5.1% difference in the RGU motif. Yet, MERS-CoV and HKU5 are clearly two distinct species. For accommodating these situations, Drexler *et al*. (2014) revised the RGU threshold for betacoronaviruses to 5.1%. In addition to the above full-length or partial genomic sequences, there are hundreds of additional partial RdRp sequences deposited in GenBank. These sequences are shorter than 816 nt and remain as yet unclassified.

5.4 CONCLUSIONS

A number of conclusions can be drawn based on our current knowledge of bat coronaviruses:

1. Bats carry a great genetic diversity of coronaviruses and are probably the natural and ancestral reservoirs of alphacoronaviruses and betacoronaviruses. Some of these viruses have evolved to infect other species.
2. Diverse SL-CoVs are circulating in horseshoe bats and one of them (the prototype of SARS-CoV) was transmitted to civets through S gene mutations, resulting in the 2002–2003 SARS pandemic. Some bat SL-CoVs such as WIV1 can use the cellular ACE2 receptors of humans, civets, and bats, suggesting the potential for direct transmission from bats to other animals including humans.
3. Bats are likely natural reservoirs of MERS-CoV or an ancestral MERS-like CoV. Future investigation of MERS-CoV and its origins should focus on bats of the following families: Molossidae, Vespertilionidae, Nycteridae, and Emballonuridae.
4. From 55 published articles on bat coronaviruses at the time of preparation for this book chapter, more than 102 bat species from around the world have been shown to carry coronaviruses (Chen *et al.*, 2014). Currently, eight bat coronaviruses have been classified as species, but more than one hundred bat coronaviruses (or strains) have not yet been classified. However, as there are more than 1200 bat species in the world, large numbers of new bat coronaviruses likely await discovery.
5. Bat coronaviruses have not been fully discovered due to the diversity of bats across the globe. Through the lesson of SARS and MERS, the transmission of animal coronaviruses to humans can be expected to continue. To address public health concerns regarding coronaviruses, strategies should be properly developed for rapid diagnosis and evaluation of the ability for cross-species transmission of animal, and particularly bat, coronaviruses.

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